POSSIBLE DEFENSE MOLECULES IN BARLEY

F. García-Olmedo

Bioquímica y Biología Molecular
E.T.S. Ingenieros Agrónomos-UPM
E-28040 Madrid, Spain

INTRODUCTION

The recent development of refined molecular tools to study gene structure and function, as well as the availability of genetic transformation methods for all kinds of organisms, including plants, has lent new vigor to the investigation of plant defense mechanisms, a subject with a long and distinguished tradition.

A variety of approaches are being followed by molecular biologists to analyze plant responses to pathogens and pests. Three of these emerge as predominant: i) **From disease to relevant molecules**, a strategy that involves challenging a plant with a pathogen or pest, identifying the plant genes that are switched on in response to the challenge, and attempting to correlate the functional properties of at least some of these genes with a defense role. ii) **From disease resistance to relevant molecules**, an approach based on finding cosegregation of a resistance gene and certain molecular features in a near-isogenic background. iii) **From molecules with toxic or deterrent properties to enhanced resistance**, an approximation which implies the construction of an agronomic trait out of the known activities of appropriate molecules and their corresponding genes.

It is not pertinent here to discuss the relative merits of these strategies, which are not mutually exclusive. My aim is rather to examine to what extent they have allowed the identification of possible defense molecules in barley and related species. Because of circumstantial and/or objective reasons, monocots, in general, and barley, in particular, have not been the plant systems where some of the original studies have been carried out. However, for obvious reasons a significant part of recent interest is focusing on them. Plant defense mechanisms are still far from being elucidated (see Bowles 1990), so this non-comprehensive review will have the more modest objective of summarizing some relevant information concerning possible defense molecules in barley and its close relatives.

TYPES OF MOLECULES

Types of molecules whose *in vitro* properties and/or physiological behaviour suggest possible involvement in defense and are present in barley or in closely-related species are listed in Table I. Most of these, but not all, are proteins that show toxic or inhibitory activities towards heterologous systems or whose genes are induced when challenged with pathogens or pests.

Pathogenesis-related (PR) proteins induced in plants infected with pathogens

Table I. Possible defense molecules in barley

Type	Variants	Active against	Location
Chitinases	C	fungi	Endosperm
	T	"	Aleurone
	K	"	Cell suspension
	PR3		Leaves
Glucanases	GI-GVI	fungi	Endosperm Leaves Cell suspension Roots
RIP	>1	ribosomes fungi	Endosperm
Inhibitors	BASI	subtilisin α-amylase endogenous	Endosperm
	CI-1	chymotrypsin	Endosperm
	CI-2	subtilisin	"
	PR5	?	Leaves
	BMAI-1 BDAI-1 BTAI-CMa BTAI-CMb BTAI-CMd	monomeric, dimeric, tetrameric	Heterologous α-amylases (insects)	Endosperm
	BTI-CMc BTI-CMe	trypsin		Endosperm
Thionin I	α β	bacteria and fungi		Endosperm
Thionin II	DB4 DG3 DG4	bacteria and fungi		Leaves
Thionin V	TTHV	?		Endosperm
PR Proteins	PR1 & others			Leaves
Lectins	?			Germ ?
Hydroxamic acids				Ubiquitous

or treated with chemicals, are extractable at low pH and predominantly appear in the intracellular spaces (see Van Loon, 1985). These proteins have been most intensively studied in dicots and only recently have they been identified in monocots and, more specifically, in barley, where proteins corresponding to PR1 and PR5 from tobacco have been found (White *et al.* 1987; Bryngelson *et al.* 1988; Bryngelson and Green 1989). Up to ten PR proteins have been isolated from maize,four of which have been identified as chitinases and two as 1-3-β-glucanases (Nasser *et al.* 1988, 1990). These hydrolases have been reported to inhibit fungal growth *in vitro* (Schlumbaum *et al.* 1986; Mauch *et al.* 1988). Barley and wheat have both chitinases (Roberts and Selitrennikoff 1986; Leah *et al.* 1987, 1991; Broekaert *et al.* 1988; Swegle *et al.* 1989; Jacobsen *et al.* 1990; Ride and Barber 1990; Kragh *et al.* 1991) and 1,3-β-glucanases (Ballance amd Svendsen 1988; Høj *et al.* 1988, 1989; Sock *et al.* 1990; Kragh *et al.* 1991; Leah *et al.* 1991) in various tissues, specially in endosperm, and under different physiological situations.

A complex class of possible defense-related proteins is represented by plant proteinaceous inhibitors of proteases and α-amylases of heterologous systems (see García-Olmedo *et al.* 1987). These were classified into 11 families or types of which more than half were represented in cereals. The activity *in vitro* and *in vivo* of some of these inhibitors versus insect enzymes and whole insects has been recently reviewed (García-Olmedo *et al.* 1991).

The thionins are 5 kDa polypeptides, which are cysteine-rich and whose toxicity to plant pathogens was reported long ago (Fernandez de Caleya *et al.* 1972). Different structural types and several genetic variants of each have been described in barley, as well as in other taxa (see García-Olmedo *et al.* 1989, 1991, 1992). A 30 kDa ribosome-inactivating protein (RIP) from barley, related to ricin and tritin, has antifungal activity (Asano *et al.* 1986; Leah *et al.* 1991).

Some possible defense proteins characterized in wheat have not yet been identified in barley. This is the case, for example, of wheat germ agglutinin (Smith and Raikhel 1989; Huesing *et al.* 1991) and a pathogen-induced protein which is homologous to glutathione-S-transferase (Dudler *et al.* 1991).

No evidence of phytoalexins exhibiting a defense function in cereals is available, but in contrast, lignification seems to play a key role in the hypersensitive reaction (Moerschbacher *et al.* 1990). Thus, specific suicidal inhibitors of the lignification pathway, applied prior to inoculation of resistant wheat with stem rust, decreased the frequency of necrotic host cells and led to increased fungal growth (Moerschbacher *et al.* 1990).

Among non-protein defense-related molecules in cereals, hydroxamic acids have received particular attention (see Niemeyer 1988). These compounds are active against pathogens and other organisms, but an unequivocal link to defense mechanisms *in vivo* is still lacking (Niemeyer 1988).

Cloning of cDNAs corresponding to mRNAs induced during infection is an approach which is actively pursued at present, as, for example, in the resistant barley/powdery mildew interaction (Davidson *et al.* 1987).

The more relevant types of molecules alluded to above will be discussed in the following paragraphs, with special emphasis on their plant-defense properties *in vitro* and/or *in vivo*.

CHITINASES AND 1,3-β-GLUCANASES

A 28 kDa protein from barley endosperm (protein C) with antifungal properties (Roberts and Selitrennikoff 1986) was subsequently found to be an endochitinase (Leah *et al.* 1987). A closely similar one was identified in barley aleurone (Swegle *et al.* 1989), which was later identified as the antifungal basic chitinase T. It is a 33 kDa protein with a 23 amino acid extension at the N-terminal domain that was not present in chitinase C and was homologous (73% identity) with the B domain of wheat germ lectin and the N-terminal of a chitinase from bean leaves (Jacobsen *et al.* 1990). What seems to be an isoform of chitinase C, designated CHI26 has been recently reported by Leah *et al.* (1991). Suspension cultures of barley secrete chitinases T and C and a third chitinase, designated K, which is also present in the barley grain (Kragh *et al.* 1991). The first chitinase, PR3, has been described in barley leaves (Bryngelson *et al.* 1991), and four major forms have been recently purified from wheat leaves (Ride and Barber 1990). Two 1,3-β- endoglucanases, designated GI and GII, have been purified and their corresponding cDNAs cloned from barley seeds (Høj *et al.* 1988, 1989; Ballance and Svendsen 1988; Leah *et al.* 1991). One of them, GII, has been identified among secreted proteins in suspension cultures (Kragh *et al.* 1991). More recently, up to six 1,3-β-endoglucanases (GI-GVI) have been identified and their differential expression pattern has been studied (Slakeski *et al.* 1991). No investigation of their induction in barley leaves seems to be available, but these enzymes have been shown to be elicited in wheat leaves both abiotically and by stem rust infection (Sock *et al.* 1990).

In line with previous observations with other chitinases and 1,3-β-glucanases (Mauch *et al.* 1988), Leah *et al.* (1991) have shown synergism between the two types of barley enzymes in their inhibitory activity against fungal pathogens *in vitro*. Furthermore, they have shown synergism between these enzymes and the ribosome-inactivating protein.

PROTEINACEOUS INHIBITORS OF PROTEASES AND α-AMYLASES

Inhibitor families

The Kunitz (STI) family includes inhibitors of serine proteinases and endogenous α-amylases (see García-Olmedo *et al.* 1987) and is represented in barley by an inhibitor, designated BASI, which inhibits subtilisin and type-2 endogenous α-amylase (Mundy *et al.* 1984; Svendsen *et al.* 1986).

Double-headed and single-headed trypsin inhibitors from wheat germ (Odani *et al.* 1986) are homologous to the well characterized double-domain Bowman-Birk inhibitors from the *Leguminosae*, which inhibit trypsin and chymotrypsin. Occurrence of these inhibitors in barley germ has not been demonstrated, but can be considered as likely.

Two inhibitors of barley endosperm, CI-1 and CI-2, which are homologous to the potato inhibitor I, have been well characterized (Jonassen and Svendsen 1982). Both of them inhibit chymotrypsin and subtilisin, but CI-1 has a single reactive

site for the two enzymes and CI-2 has one site for chymotrypsin and two for subtilisin.

Bryngelson and Green (1989) have characterized a pathogenesis-related, thaumatin-like protein, designated PR-5 which is induced in barley leaves when challenged with an incompatible race of mildew. This protein is homologous with PR-5 from tobacco and is also related to an inhibitor of porcine pancreatic α-amylase from *Eleusine coracana* (Campos and Richardson 1984) and to the sweet protein thaumatin II. A second barley protein, designated PAPI, is more distantly related (Svensson *et al.* 1986).

The cereal trypsin/α-amylase inhibitor family is perhaps the most diversified among those in barley (Table I), as it includes both trypsin inhibitors and the subunits of monomeric, dimeric and tetrameric inhibitors of heterologous α-amylases (see García-Olmedo *et al.* 1987, 1991, Sanchez-Monge *et al.* 1988). These inhibitors are encoded by a multigene family which is dispersed over several chromosomes in both wheat and barley.

Defense properties of the inhibitors

It is difficult to speculate about the natural targets for the rich arsenal of potential weapons represented by the above inhibitors. The last family's activity against the α-amylases from insects suggested long ago its possible role in pest control. Thus, monomeric and dimeric α-amylase inhibitors show different specificity versus the α-amylases from human saliva and from the digestive tract of the insect *Tenebrio molitor*. The tetrameric inhibitors seem to be active against the α- amylase from the insect but not from the salivary (Sanchez-Monge *et al.* 1986; Gomez *et al.* 1989). Furthermore, different insect α-amylases are discriminated by the inhibitors: i.e. the enzyme from *T. molitor* is more sensitive to monomeric than to dimeric types and the opposite is true for the origine from *Leptinotarsa decemlineata* (colorado potato beetle); still for other insect enzymes, both types of inhibitors are about equally effective (Gutierrez *et al.* 1990).

Insects that are able to feed on cereal endosperm have unusually high levels of α-amylase (Silano *et al.* 1975; Gutierrez *et al.* 1990). Other insects, such as *Callosobruchus maculatus* are sensitive to low inhibitor concentrations in the diet (Gatehouse *et al.* 1986). More recently, transgenic tobacco plants carrying chimeric genes encoding α-amylase and trypsin inhibitors from this family have been found to be lethal to *Agrotis ipsilon* and *Spodoptera littoralis* in a leaf-disc assay (Carbonero *et al.* unpublished).

THIONINS

Types present

The thionins are polypeptides of about 5 kDA, which have three or four disulphide bridges and are toxic to plant pathogens (García-Olmedo *et al.* 1989, 1991a,b). Out of five structural thionin types into which all the known sequences of this family can be classified (García-Olmedo *et al.* 1992), three of them (I, II, V) are represented by one or more genetic variants in barley and wheat. Type I

corresponds to the original endosperm thionins. Thionins of this type have four disulphide bridges and are highly basic (see García-Olmedo *et al.* 1992). Type II correspond to the leaf thionins, which are structurally very similar to those of type I (Gausing 1987; Bohlman and Apel 1987). Type V thionins have three disulphide bridges and very few charged amino acids. They are also in the endosperm (A. Castagnaro, unpublished).

Thionins of types I and V accumulate in endosperm during the first half of its developmental period, while those of type II are synthesized in ethiolated leaves or in green leaves under stress conditions (Bohlman *et al.* 1988; Ebrahim-Nesbat *et al.* 1989).

Defense properties of thionins

The toxicity of thionins towards different kinds of organisms and to cells in culture has been investigated for several decades (see García-Olmedo *et al.* 1991a,b). Plant pathogenic bacteria of the genera *Pseudomonas, Xanthomonas, Agrobacterium, Erwinia* and *Corynebacterium* are sensitive to thionin (Fernandez de Caleya *et al.* 1972 and unpublished). In a recent survey, sensitivity of fungal pathogens to pure genetic variants was in the 10^{-6} M-10^{-5} M range (García-Olmedo *et al.* 1991a,b; Molina and Fraile, unpublished). We have obtained transgenic tobacco plants constitutively expressing the α-thionin gene from barley. These plants show enhanced resistance to the bacterial pathogens *Pseudomonas solanacearum* and *P. syringae* pv. tabaci.

ACKNOWLEDGEMENTS

This work was supported by the Fundación Ramón Areces and by Grants BIO90-0084 and BIO88-0216 from the Plan Nacional de Investigación Científica y Técnica.

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